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SEMI-ANNUAL PROGRESS REPORT

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July 1, 1952
For period 1 January 1952
to 1 July 1952

Contractor: The Johns Hopkins University
NR: N-ONR-248(15)
Contract: HG 11.38
Annual Rate: $8,000
Principal Investigator: Thomas G. Ward, M.D.
Assistants: Frederick A. H. Rice, Ph.D.
A. George Hubbard, Jr. A.B.
Title of Project: Antigenic Studies on Influenza Virus

Objectives:

To improve immunization against influenza by studing fundamental relationships between cells and virus.

Abstract of Results:

A. Previous to present period:

1. Monovalent influenza virus vaccines were used in a study at Great Lakes where indications were secured that the vaccinated individuals had a slight reduction in the total respiratory disease rate. No influenza occurred during the winter of 1948-1949 and, therefore, the results cannot be evaluated from the point of view of prevention of influenza.

2. Fourteen different strains of influenza virus were checked homologously and heterologously by the following tests:

   a. Hemmagglutination inhibition.
   b. Mouse neutralization, using live virus as a vaccine.
   c. Mouse neutralization, using dead virus as a vaccine.
   d. Antibody producing capacity test (Eddy).
   e. Egg neutralization test.
   f. Complement fixation test.

   This study indicated that the Thompson strain in influenza virus had the greatest spread of "protectiveness" but that its "invasiveness" was similar to other A strains of virus employed in the protocols.

3. A crystalline receptor substance has been isolated from human lungs, beef lungs and pork lungs. This material contains carbon, hydrogen, oxygen, nitrogen and phosphorus. It is in the crystalline state and the original weight of lung yields about 10^{-5} grams of the final product. It will inhibit a hemagglutination by influenza virus in the order of 0.01 gamma.

4. Chemical studies, using ionic exchange columns and chromotography, have been utilized in an attempt to understand the chemical nature of the crystalline receptor substance Antigenic studies carried out during the report period prior to this one have indicated that the material is very likely not antigenic. The concepts of developing a possible inhibitor antiserum, which would combine with the receptor sites of susceptible cells, has had to be reconsidered. The negative character of these findings cannot be considered conclusive, as studies utilizing adjuvents have not been completed.

B. Activities during the present period:

1. The studies on further chemical characterization of the crystalline receptor substance are progressing. The supply of the receptor substance was jeopardized by the gasoline strike which cost us approximately two months delay in time. The material degenerated in an air freight depot in New York before it arrived in Baltimore.

The effect on activity of the receptor substance has been studied by using a reaction mixture consisting of live influenza virus and the receptor substance. These studies have demonstrated that the influenza virus is capable of destroying the activity of the receptor material. Inorganic phosphorus is promptly released in the reaction mixture.

2. In order to understand the relation between the virus particle and the cell, the model system of influenza virus and the chicken red blood cell has been utilized, using radioactive phosphorus as an indicator system.

If one adsorbs and elutes Lee influenza virus from normal chicken red blood cells and treats other red blood cells with normal allantoic fluid under similar laboratory conditions, and then adds to each tube inorganic radioactive phosphorus, he finds that the virus treated cells are capable of picking up more of the radioactive phosphorus than the cells treated with normal allantoic fluid. Interpretation of this data is somewhat difficult at the moment, but apparently the virus alters the surface of the red cell in such a way that a more rapid exchange of inorganic phosphorus is taking place between the virus treated cell and the inorganic phosphorus in the substrate than is true for the normal control cell.

On the other hand, if one labels the red blood cell in the chick embryo by inoculation of radioactive phosphorus and then treats those red blood cells with virus and with normal allantoic fluid, he finds that there is no difference between the release of phosphorus in the virus treated and the control cells.

A third variable which has been carried out with respect to this kind of experiment, has been to label the influenza virus with radioactive phosphorus and test this radioactive virus against normal chicken red blood cells. The control in this instance has been allantoic fluid harvested from embryos which had received radioactive phosphorus, but had not been inoculated with virus. In this instance, the specific activity of the red blood cells in the two systems (virus and control) are the same.

3. Another series of experiments attempting to understand the relation of the virus and the cell have been carried out using purified influenza virus which had been labelled with radioactive phosphorus. The labelling is accomplished by inoculation of the chick embryo with radioactive phosphorus about 48 hours prior to allantoic inoculation of the virus suspension. After approximately 40 hours of incubation, the eggs are chilled, the allantoic fluid harvested, and the virus purified by adsorption onto and elution from chicken red blood cells. The resultant supernatant is further purified by high speed centrifugation. The pellet is then washed with a phosphate buffer until the amount of inorganic phosphorus in the virus has been reduced to a minimum. The preparation is then placed in a growing tissue culture and the change from organic phosphate to inorganic phosphate is studied with time. Apparently the organic phosphate becomes inorganic phosphate very rapidly. The only logical explanation for these observations is that the virus disintegrates rapidly on entrance into a susceptible cell. It must be emphasized that these results are highly preliminary and need not only quantitation, but replication.

Plans for Future:

Immediate: The immediate plans include further studies on the chemical structure of the receptor substance; continued efforts to purify the influenza virus; attempts to quantitate the red blood cell virus radioactive phosphorus mixture studies; and attempts to understand what happens to the virus when it enters a susceptible cell, using radiochemical methods.

Long Range: The long range plans are concerned primarily with an attempt to understand the mechanism of the invasion of cells by influenza virus, with a subsequent hope of developing some method for interfering with this phenomenon.

Reports and Publications:

There were no publications during the current report period.